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08/951,733
A-433B

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 48/00, C07H 21/04 — A1

(11) International Publication Number: **WO 97/37691**

(43) International Publication Date: 16 October 1997 (16.10.97)

(21) International Application Number: PCT/US97/05773

(22) International Filing Date: 8 April 1997 (08.04.97)

(30) Priority Data:
08/630,242 10 April 1996 (10.04.96) US

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: TELOMERASE INHIBITORS

(57) Abstract

The invention provides oligonucleotide N3' - > P5' phosphoramidates for inhibiting telomerase activity. In the preferred method of the invention, inhibition is achieved by contacting a telomerase with oligonucleotide N3' - > P5' phosphoramidates complementary to the telomere binding region of the RNA component of the telomerase ribonucleoprotein. The nuclease resistance and strong RNA binding characteristics of N3' - > P5' phosphoramidates provide extremely efficient inhibition of telomerase activity. Telomerase inhibitors of the invention are useful as highly specific growth inhibitors of an extremely wide range of cells and organisms, including but not limited to, cancer cells, fungus, protozoans, and the like.

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TELOMERASE INHIBITORS

Field of the Invention

The invention relates generally to methods and compositions for inhibiting cell proliferation, and more particularly, to methods and compositions for inhibiting telomerase activity, particularly human telomerase activity, by contacting a telomerase with an oligonucleotide N3'->P5' phosphoramidate.

Background

Telomerase is a species-specific ribonucleoprotein that catalyzes the addition of oligonucleotide repeats onto the ends of chromosomal DNAs, i.e. telomeres, to compensate for losses that occur with each round of DNA replication, e.g. Rhyu, J. Natl. Cancer Inst., 87: 884-894 (1995). Somatic cells have little or no telomerase activity and stop dividing when the telomeric ends of at least some chromosomes have been shortened to a critical length. In contrast, in immortal cells and proliferatively active cells, there is a strong correlation between telomerase activity and proliferation or cell immortality, e.g. Hiyama et al, J. Natl. Cancer Inst., 87: 895-902 (1995); Hiyama et al, Nature Med., 1: 249-255 (1995); Kim et al, Science, 266: 2011-2015 (1994); and the like. These observations have led to the suggestion that telomerase inhibitors may be useful as highly specific agents to treat conditions associated with uncontrolled or undesired cell proliferation, such as cancers, parasitic infections, fungal infections, and the like, e.g. Morin, J. Natl. Cancer Inst., 87: 859-861 (1995); Rhyu (cited above); West et al, International patent application PCT/US94/13122; and the like. Several approaches have been suggested for obtaining such inhibitors, including screening for small molecule inhibitors, such as nucleotide triphosphate analogs, that inhibit the reverse transcriptase activity of the telomerase, and the use of inhibitory, or "antisense," oligonucleotides that are capable of forming a duplex with the RNA moiety of the telomerase, e.g. West et al (cited above) and Rhyu (cited above). Although inhibitory oligonucleotides have been used in experimental systems with positive results (e.g., Greider et al, Nature, 337: 331-337 (1989)), many of the same problems associated with the use of oligonucleotides as antisense therapeutics also exist for the use of oligonucleotides as telomerase inhibitors. Such problems include susceptibility to nuclease digestion, lack of solubility, lack of specificity, delivery, and the like, e.g. Stein et al, Science, 261: 1004-1012 (1993) and Wagner, Nature, 372: 333-335 (1994). Furthermore, the relatively short telomere binding region of the RNA component of a telomerase, e.g. 11 ribonucleotides for human, means that unless the compounds employed are enhanced for duplex forming ability, telomerase inhibition is likely to be achieved only at concentrations that produce unacceptable side-effects.

In view of the above, successful telomerase inhibition could be achieved if oligonucleotide analogs were available that not only possessed adequate nuclease resistance and solubility, but also that could form stable short duplexes with RNA under physiological conditions.

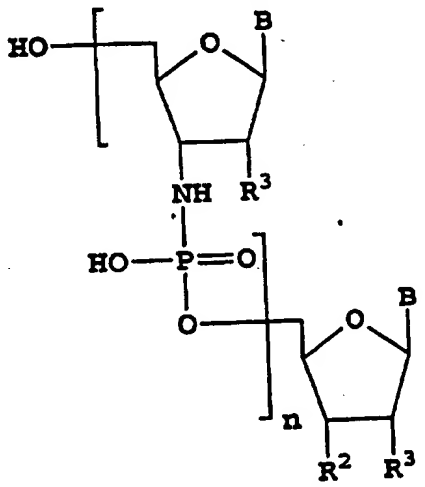
Summary of the Invention

An object of the invention is to provide a method and compounds for inhibiting telomerase activity.

Another object of the invention is to provide methods and compounds for treating conditions or diseases associated with increased or inappropriate telomerase activity, such as cancers and various parasitic infections involving eukaryotic organisms.

Yet another object of the invention is to provide oligonucleotide compounds for forming stable duplexes with the telomere binding region of the RNA moiety of telomerases, particularly human telomerase.

These and other objects of the invention are accomplished by providing oligonucleotide N3'->P5' phosphoramidates with sequences complementary to the telomere binding region of the RNA moiety of the telomerase to be inhibited. Preferably, the oligonucleotide N3'->P5' phosphoramidates of the invention have the following formula:



wherein B is a purine, pyrimidine, or an analog thercof; R^3 is hydrogen or fluoro; R^2 is hydroxyl or amino; and n is between 5 and 19.

Brief Description of the Drawings

Figure 1 illustrates a scheme for synthesizing a first type uracil monomer for assembling oligo-2'-fluoronucleoside N3'->P5' phosphoramidates of the invention.

Figure 2 illustrates a scheme for synthesizing a second type uracil monomer for assembling oligo-2'-fluoronucleoside N3'->P5' phosphoramidates of the invention.

Figure 3 illustrates a scheme for synthesizing cytosine monomers for assembling oligo-2'-fluoronucleoside N3'->P5' phosphoramidates of the invention.

Figure 4 illustrates a scheme for synthesizing oligo-2'-fluoronucleoside N3'->P5' phosphoramidates of the invention.

Figure 5 illustrates a scheme for synthesizing oligo-2'-deoxynucleotide N3'->P5' phosphoramidates.

Definitions

Whenever an oligonucleotide is represented by a sequence of letters, such as "ATGUCCTG," it will be understood that the nucleotides are in 5'->3' order from left to right and that "A" denotes deoxyadenosine, "C" denotes deoxycytidine, "G" denotes deoxyguanosine, "T" denotes thymidine, and "U" denotes deoxyuridine, unless otherwise noted.

As used herein, "N3'->P5' phosphoramidate" refers to an internucleosidic linkage of the form:

3'-NH-P(=O)(OR^4)-O- 5'

wherein the 3' and 5' refer to the carbon atoms of the sugar moieties of consecutive nucleosides which are connected by way of the linkage, and wherein R^4 is hydrogen or a phosphate protecting group. More particularly, R^4 may by alkyl, alkenyl, aryl, aralkyl, or cycloalkyl containing up to 10 carbon atoms. Preferably, R^4 is alkyl having from 1 to 6 carbon atoms; electron- withdrawing β-substituted ethyl, particularly β-trihalomethyl-, β-cyano-, β-sulfo-, β-nitro- substituted ethyl, or the like; electron-withdrawing substituted phenyl, particularly halo-, sulfo-, cyano-, or nitro-, substituted phenyl; or electron-withdrawing substituted phenylethyl. More preferably, R^4 is methyl, β-cyanoethyl, or 4-nitrophenylethyl. Most preferably, R^4 is hydrogen. Electron-withdrawing substituents are typically halo, cyano, nitro, sulfo, or mono-, di-, or trihalomethyl, and the like. Halogen atom substituents are usually fluoro, chloro, bromo, or iodo; and preferably, they are fluoro or chloro. "Electron- withdrawing" denotes the tendency of a substituent to attract valence electrons of the molecule of which it is a part, i.e. it is electronegative, e.g. March, Advanced Organic Chemistry, pgs. 16-18 (John Wiley, New York, 1985). For convenience, nucleotide phosphoramidates are sometimes indicated herein by a subscripted "np" or "pn" for N3'->P5' phosphoramidates and P3'->N5' phosphoramidates, respectively. Thus, "$U_{np}U$" is a dinucleotide in which a 3'-aminouridine and a deoxyuridine are linked by an N3'->P5' phosphoramidate linkage. Similarly, 2'-fluoro substituents are indicated by a superscripted "f". Thus, "$U^f_{np}U$" is a dinucleotide in which the 3'-most 2'-fluorouridine is linked to a

uridine by an N3'->P5' phosphoramidate linkage. A single leading subscripted "p" indicates to a 5' monophosphate, and a single trailing subscripted "n" indicates a 3'-amino.

As used herein, "nucleoside" includes the natural nucleosides, including 2'-deoxy and 2'-hydroxyl forms, e.g. as described in Kornberg and Baker, DNA Replication, 2nd Ed. (Freeman, San Francisco, 1992). "Analogs" in reference to nucleosides includes synthetic nucleosides having modified base moieties and/or modified sugar moieties, e.g. described generally by Scheit, Nucleotide Analogs (John Wiley, New York, 1980). Such analogs include synthetic nucleosides designed to enhance binding properties, e.g. stability, specificity, or the like, such as disclosed by Uhlmann and Peyman, Chemical Reviews, 90: 543-584 (1990).

As used herein, "pyrimidine" means the pyrimidines occurring in natural nucleosides, including cytosine, thymine, and uracil, and common analogs thereof, such as those containing oxy, methyl, methoxy, hydroxyl, amino, thio, 5-propynyl, and like, substituents. The term as used herein further includes pyrimidines with common protection groups attached, such as 4N-benzoylcytosine. Further common pyrimidine protection groups are disclosed by Beaucage and Iyer, Tetrahedron, 48: 2223-2311 (1992).

As used herein, "purine" means the purines occurring in natural nucleosides, including adenine, guanine, and hypoxanthine, and common analogs thereof, such as those containing oxy, methyl, methoxy, hydroxyl, amino, thio, and like, substituents. The term as used herein further includes purines with common protection groups attached, such as 2N-benzoylguanine, 2N-isobutrylguanine, 6N-benzoyladenine, and the like. Further common purine protection groups are disclosed by Beaucage and Iyer (cited above).

As used herein, "telomere binding region" means the segment of the RNA moiety of a telomerase that serves as a template for the addition of telomeric repeat units by the telomerase. The sequence and length of such regions vary according to species, and for humans is described in Feng et al, Science, 269: 1236-1241 (1995). Such regions may be determined using the techniques described in Feng et al for determining the sequence of the human telomere binding region.

Detailed Description of the Invention

The present invention is directed to methods and pharmaceutical compositions comprising oligonucleotide N3'->P5' phosphoramidate compounds for inhibiting telomerase activity, particularly human telomerase activity. Preferably, for treating human cancers, a oligonucleotide phosphoramidate compound of the invention has from 6 to 15 nucleotides and includes sequence complementary to the telomere binding region of the RNA moiety of the telomerase. More preferably, such a compound has a length of between 6 and 11 nucleotides. Most preferably, such a compound completely complementary to the telomere binding region of the RNA moiety of the telomerase.

Preferred lengths for inhibiting telomerases of other species depend in part on the lengths of the respective telomere binding regions of the RNA moieties of those species. Generally, however, the length of an oligonucleotide N3'->P5' phosphoramidate compound will be between 6 and 20 nucleotides in length, even if a telomere binding region of the RNA moiety is longer. More preferably, such compounds are between 8 and 15 nucleotides in length.

The nucleotide sequence selected for the oligonucleotide N3'->P5' phosphoramidate compounds depends on the nature of the cell type whose proliferation is sought to be inhibited. For human cancers, the nucleotide sequence of compounds of the invention preferably contain or comprise a subset of the following 11-mer:

5'-GTTAGGGTTAG-3'

which is the complement to the telomere binding region of the RNA moiety of human telomerase. Thus, preferred 6-mer to 11-mer sequences for treating human cancers include 5'-GTTAGGGTTAG, 5'- GTTAGGGTTA, 5'- TTAGGGTTAG, 5'- GTTAGGGTT, 5'- TTAGGGTTA, 5'- TAGGGTTAG, 5'- GTTAGGGT, 5'- TTAGGGTT, 5'- TAGGGTTA, 5'- AGGGTTAG, 5'- GTTAGGG, 5'- TTAGGGT, 5'- TAGGGTT, 5'- AGGGTTA, 5'- GGGTTAG, 5'- GTTAGG, 5'- TTAGGG, 5'- TAGGGT, 5'- AGGGTT, 5'- GGGTTA, and 5'- GGTTAG. For candida fungus, telomerase RNA moieties are disclosed by West et al, International patent application PCT/US94/13122.

For inhibiting telomerases whose RNA moieties need to be determined, Morin, Cell, 59: 521-529 (1989); West et al (cited above); Feng et al, Science, 269: 1236-1241 (1995), and others, disclosed techniques for determining RNA components of telomerases. Once such sequences are determined, appropriate oligonucleotide N3'->P5' phosphoramidate compounds are readily selected.

Synthesis of Oligonucleotide N3'->P5' Phosphoramidates

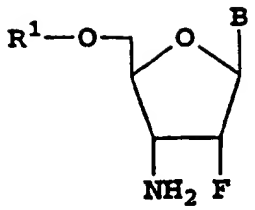
Synthesis of oligonucleotide N3'->P5' phosphoramidates is disclosed in the following publications: Gryaznov et al, International patent application PCT/US95/03575; Gryaznov et al, J. Am. Chem. Soc., 116: 3143-3144 (1994); Gryaznov et al, Proc. Natl. Acad. Sci., 92: 5798-5802 (1995); and Chen et al, Nucleic Acids Research, 23: 2661-2668 (1995). Briefly, oligonucleotide N3'->P5' phosphoramidates are synthesized on a solid support using the step-by-step elongation procedure outlined in Figure 5. The synthetic cycle for addition of a single aminonucleoside consists essentially of the following operations: detritylation (step a); phosphitylation of the 5' hydroxyl group to generate a 5'-hydrogen phosphonate diester (steps b and c); and Atherton-Todd type coupling of a 5'-

DMT-3'-aminonucleoside (e.g. as disclosed by Glinski et al, Chem. Comm., pp. 915-916 (1970)) with the 5' hydrogen phosphonate in the presence of carbon tetrachloride (step d). Coupling yields range between 94-96% per cycle. The resulting oligonucleotide phosphoramidate is cleaved and deprotected with ammonia and thereafter purified by ion exchange high performance liquid chromatography. The following references provide further guidance for carrying out the above synthesis: Atherton et al, J. Chem. Soc., pp. 660-663 (1945); Gryaznov et al, Nucleic Acids Research, 20: 3403-3409 (1992); Gryaznov et al, Vest. Mosk. Univ. Ser. 2: Khim 27: 421-424 (1986); and Gryaznov et al, Tetrahedron Lett., 31: 3205-3208 (1990).

A wide variety of solid phase supports may be used in the synthesis of oligonucleotide N3'->P5' phosphoramidate (2'-deoxy and 2'-fluoro) compounds, including microparticles made of controlled pore glass (CPG), highly cross-linked polystyrene, acrylic copolymers, cellulose, nylon, dextran, latex, polyacrolein, and the like, disclosed in the following exemplary references: Meth. Enzymol., Section A, pages 11-147, vol. 44 (Academic Press, New York, 1976); U.S. patents 4,678,814; 4,413,070; and 4,046;720; and Pon, Chapter 19, in Agrawal, editor, Methods in Molecular Biology, Vol. 20, (Humana Press, Totowa, NJ, 1993). Supports further include commercially available nucleoside-derivatized CPG and polystyrene beads (e.g. available from Applied Biosystems, Foster City, CA); polystyrene grafted with polyethylene glycol (e.g., TentaGel™, Rapp Polymere, Tubingen,Germany); and the like. Selection of the support characteristics, such as material, porosity, size, shape, and the like, and the type of linking moiety employed depends on a variety of factors, such as protection groups employed, length of final product, quantity of final product, and the like. Exemplary linking moieties are disclosed in Pon et al, Biotechniques, 6:768-775 (1988); Webb, U.S. patent 4,659,774; Barany et al, International patent application PCT/US91/06103; Brown et al, J. Chem. Soc. Commun., 1989: 891-893; Damha et al, Nucleic Acids Research, 18: 3813-3821 (1990); Beattie et al, Clinical Chemistry, 39: 719-722 (1993); Maskos and Southern, Nucleic Acids Research, 20: 1679-1684 (1992); and the like.

Synthesis of Oligo-2'-fluoronucleotide N3'->P5' Phosphoramidates

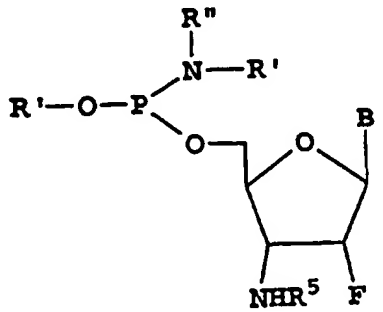
Oligo-2'-fluoronucleotide N3'->P5' Phosphoramidates for use in the invention may be synthesized by several different approaches. In a first approach, coupling is achieved by carbon tetrachloride-driven oxidative phosphorylation of a nucleoside 3'-amine by a 5'-H-phosphonate of the terminal nucleotide of a growing chain anchored to a solid phase support, as described, for example, by Gryaznov et al, J. Am. Chem. Soc.116: 3143-3144 (1994); and Chen et al, Nucleic Acids Research, 23: 2661-2668 (1995); which references are incorporated by reference. Monomers for this synthetic approach have the following form:



where B is defined as above and R1 is hydrogen or a hydroxyl protecting group, such as triphenylmethyl (i.e., trityl), p-anisyldiphenylmethyl (i.e., monomethoxytrityl or MMT), di-p-anisylphenylmethyl (i.e., dimethoxytrityl or DMT), pivaloyl, acetyl, 4-methoxytetrahydropyran-4-yl, tetrahydropyranyl, phenoxyacetyl, isobutyloxycarbonyl, pixyl, benzyl, trialkylsilyl having from 3 to 9 carbon atoms, 9-fluorenylmethyl carbamate (Fmoc), or the like. Preferably, R1 is DMT. Greene and Wuts, Protective Groups in Organic Synthesis, 2nd Edition (John Wiley, New York, 1991) provides extensive guidance on the selection of protecting groups for the various embodiments of the invention.

A general scheme for preparing the above monomers is shown in Figure 1. Briefly, a ribonucleoside is transformed into a 5'-hydroxyl-protected-2',3'-anhydroxylnucleoside, after which the 2',3'-epoxy ring is opened by treatment with sodium azide, or like reagent, to form a 5'-hydroxyl-protected-3'-azido-3'-deoxyarabinonucleoside. After purification, the 5'-hydroxyl-protected-3'-azido-3'-deoxyarabinonucleoside is fluorinated at the 2' position by treatment with diethylaminosulfur trifluoride (DAST), or like reagent, after which the azido group is reduced to give the above monomer. The monomers are then employed for chain assembly essentially as described by Chen et al and Gryaznov et al (cited above).

In a second approach, oligomers of the invention are synthesized via an exchange reaction between a phosphoramidite group on a monomer and a 3'-amino of an anchored chain, as illustrated by Scheme 4 in Figure 4.. Monomers for this synthetic approach have the following form:



where B and R^1 are defined as above, R^5 is an amino protecting group, and R' and R" taken together with the nitrogen form an alkyl- or arylamino leaving group. More particularly, R' and R" taken separately each are alkyl, aralkyl, cycloalkyl, and cycloalkylalkyl containing up to 10 carbon atoms in total. Preferably R' and R" taken separately are alkyl having from 1 to 5 carbon atoms. Most preferably, R' and R" taken separately are isopropyl. R' and R" taken together form an alkylene chain containing up to 5 carbon atoms in the principal chain and a total of up to 10 carbon atoms with both terminal valence bonds of said chain being attached to the nitrogen atom to which R' and R" are attached; or R' and R" when taken together with the nitrogen atom to which they are attached form a saturated nitrogen heterocycle which may contain one or more additional heteroatoms from the group consisting of nitrogen, oxygen, and sulfur. More preferably, R' and R" taken together and with the nitrogen to which they are attached are pyrrolidino, morpholino, or piperidino. Most preferably, R' and R" taken together and with the nitrogen to which they are attached are morpholino. Preferably, R^5 is trityl, and more preferably, R^5 is 4-methoxytrityl.

Phosphoramidite monomers are prepared as follows, as illustrated in Figures 2 and 3. As above, a ribonucleoside is transformed into a 5'-hydroxyl-protected-2',3'-anhydroxylnucleoside, after which the 2',3'-epoxy ring is opened by treatment with sodium azide, or like reagent, to form a 5'-hydroxyl-protected-3'-azido-3'-deoxyarabinonucleoside. After purification, the 5'-hydroxyl-protected-3'-azido-3'-deoxyarabinonucleoside is fluorinated at the 2' position by treatment with diethylaminosulfur trifluoride (DAST), or like reagent, after which the azido group is reduced to give a 3'-amino. After suitably protecting the 3'-amino and releasing the 5'-hydroxyl protecting group, the nucleoside is phosphitylated at the 5' oxygen to give the crude phorphoramidite monomer.

The phosphoramidite monomers are used in the following synthetic cycle:

1) detritylation with acid of the 3'-amino group of nucleoside attached to a solid support through 5'-terminus;

2) tetrazole-catalyzed amidite transfer reaction between the phosphoramidite monomer and the 3'-amino group of the nucleoside on a solid support, resulting in formation of an internucleoside phosphoramidite diester group; this may be repeated with intermediate washing with acetonitrile, or like solvent, to achieve slightly higher efficiency of chain elongation;

3) oxidation of the newly formed internucleoside phosphoramidite diester into a phosphoramidate diester group with aqueous iodine, or like oxidizing agent;

4) capping of unreacted 3'-amino groups with acetic anhydride, or like capping agent.

This cycle can be repeated, resulting in oligo-2'-fluoronucleotide N3'ØP5' phosphoramidates after cleavage from the solid support and deprotection with ammonia.

Experimental Methods

Oligonucleotide N3'→P5' phosphoramidates, containing 2'-deoxy- and one or two 2'-fluoronucleosides were synthesized using the oxidative phosphorylation method on an ABI 394 synthesizer as described in Chen et al (cited above). Uniformly modified oligo-2'-fluoronucleotide N3'→P5' phosphoramidates were prepared by amidite transfer reaction on an ABI 380B synthesizer using the following protocol:

1) detritylation, 5% dichloroacetic acid in dichloromethane, 1 min
2) coupling, 0.1 M phosphoramidite 2 and 0.45 M tetrazole in acetonitrile, 3 min
3) oxidation, 0.1 M iodine in tetrahydrofuran/pyridine/water, 10/10/1, v/v/v, 1 min
4) capping, acetylation of unreacted 3'-amino groups by standard ABI capping solutions, 30 sec.

Chemical steps within the cycle were followed by acetonitrile washings and flushings with dry argon for 0.2-0.4 min. After cleavage from the solid support and deprotection with concentrated aqueous ammonia, 1-1.5 h, 55°C, oligonucleotides were analyzed and purified by IE HPLC. Oligonucleotides were desalted on Pharmacia NAP-5 or NAP-10 gel filtration columns immediately after purification and stored frozen or lyophilized at -18°C.

Preparation of the 5'-phosphorylated oligonucleotides was done upon sulfone-derivatized CPG (Gryaznov et al, Nucleic Acids Research, 21: 1403-1408 (1993)), and 5'-hydroxyl oligomers were synthesized upon oligonucleotide-succinyl CPG.

Dionex DX300 or DX500 systems were used for IE (ion exchange) analysis and purification of oligonucleotides. A Pharmacia MonoQ 10/10 column was used for analysis and purification of crude oligomers, eluted with a 2%/min gradient of 1.5 M NaCl in 10 mM NaOH. A Dionex NucleoPac PA100 column, eluted with a 1.5% per minute gradient of 1.5 M NaCl in 10 mM NaOH was used for all other IE HPLC analysis. A Hewlett Packard Hypersil ODS, 5μ column on a Waters HPLC system was used for RP HPLC, with a 1% per minute gradient of acetonitrile in 0.1 M triethylammonium acetate, pH 7.0.

NMR spectra were recorded on a Bruker DRX-400 spectrometer. Chemical shifts are reported relative to TMS, CCl_3F, and H_3PO_4, for ^1H, ^{19}F, and ^{31}P spectra, respectively.

Thin layer chromatography (TLC) was performed on Whatman polyester-backed silica gel plates with methanol/dichloromethane eluents.

Telomerase Inhibition Assays

Selection of a compound of the invention for a particular application is aided by determining its ability to inhibit telomerase activity in an in vitro assay. Such assays for a variety of species are described in the literature. Human telomerase assays are disclosed in Morin, Cell, 59: 521-529 (1989); West et al, U.S. patent 5,489,508; Kim et al, Science, 266: 2011-2015 (1994); and like references. Telomerase inhibition assays are established by

providing a standardized source of telomerase activity whose inhibition is measured upon contact with a prospective inhibitor. Assays for human telomerase inhibition are conveniently established with HeLa cells, e.g. as used by Morin (cited above), or 293 cells available from the American Type Culture Collection under accession No. CRL 1573 and described by Graham in J. Gen. Virol. 36:59-72 (1977); Virology, 77: 319-329 (1977); and Virology, 86: 10-21 (1978). Such cells are referred to herein as "source" cell lines.

Preferably, telomerase inhibition assays are either based on the telomerase assay disclosed by Morin (cited above) or on the PCR-based telomerase assay (TRAP assay) disclosed by Kim et al (cited above). The Morin assay is carried out as follows: S100 extract are prepared from a source cell line as follows. Approximately 6 x 10^8 cells used for each extract. Cells growing in suspension are collected by centrifugation for 10 min at 1800 r.p.m. (500 g) at 4°C with no brake, e.g. in a Beckman JA-10 fixed angle rotor. Cells growing in monolayer are harvested by scraping with a rubber policeman and centrifuged as above. The pellets are rinsed twice in cold PBS (2.7 mM KCl, 1.5 mM KH_2PO_4, 137 mM NaCl and 8 mM Na_2HPO_4) followed by centrifugation for 3 min at 2000 r.p.m. (570 g) at 4 °C in a swing-out rotor. The final pellet is rinsed in cold 2.3 x Hypo buffer (1 x Hypo buffer: 10 mM HEPES pH 8.0, 3 mM KCl 1 mM $MgCl_2$, 1 mM DTT, 0.1 mM PMSF, 10 U/ml of "RNASIN", and optionally, 1 µM leupeptin and 10 µM pepstatin A), centrifuged for 5 min and resuspended in 0.75 vol of 2.3 x Hypo buffer. After incubation on ice for 10 min the sample is transferred to an ice cold 7 ml Dounce homogenizer and homogenized on ice using a B pestle (25-55 µM clearance). After a further 30 min on ice the sample is centrifuged for 10 min at 10,000 r.p.m. (16,000 g) at 4°C, e.g. in a Beckman J3-13.1 swing-out rotor. One-fiftieth volume of 5 M NaCl was added and the sample was centrifuged for 1 h at 38,000 r.p.m. (100,000 g) at 4°C, e.g. in a Beckman Ti50 rotor. Glycerol is added to a final concentration of 20% and the extract aliquoted and stored at -70°C. Protein concentration in a typical extract is ≅ 4 mg/ml.

The level of telomerase activity is measured as follows: Aliquots (20 µl) of S-100 cell extract are diluted to a final volume of 40 µl containing 2 mM dATP, 2 mM dTTP, 1 mM $MgCl_2$, 1 µM $(TTAGGG)_3$ primer, 3.13 µM (50 µCi) [α-^{32}P]dGTP (400 Ci/mmol), 1 mM spermidine, 5 mM β-mercaptoethanol, 50 mM potassium acetate and 50 mM Tris-acetate (pH 8.5). The reactions are incubated for 60 min at 30°C and stopped by addition of 50 µl of 20 mM EDTA and 10 mM Tris-HCl (pH 7.5) containing 0.1 mg/ml RNase A, followed by incubation for 15 min at 37°C. To eliminate proteins, 50 µl of 0.3 mg/ml proteinase K in 10 mM Tris-HCl (pH 7.5), 0.5% SDS is added for 10 min at 37°C. Following extraction with phenol and addition of 40 µl or 2.5 M ammonium acetate and 4 µg of carrier tRNA, the DNA is precipitated with 500 µl of ethanol at -20°C. DNA pellets are resuspended in 3 µl of formamide loading dye, boiled for 1 min, chilled on ice and loaded

onto an 8% polyacrylamide -7 M urea sequencing gel and run at 1500 V for 2.5 h using 0.6 x TBE buffer. Dried gels are exposed to Kodak XAR-5 pre-flashed film at -70°C with enhancing screen. Typical autoradiograph exposures are between 2 and 7 days.

The TRAP assay may be implemented as follows: Preferably, extracts for the TRAP assays are prepared as follows: Source cells are washed once in phosphate-buffered saline, pelleted at 10,000g for 1 min at 4°C, resuspended in ice-cold wash buffer (10 mM Hepes-KOH (pH 7.5), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol], pelleted again, and resuspended at 10^4 to 10^6 cells per 20 µl of ice-cold lysis buffer [10 mM tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM phenylmethlsulfonyl fluoride, 5 mM β-mercaptoethanol, 0.5% CHAPS (Pierce), 10% glycerol]. The suspension is incubated 30 min in a microultracentrifuge (100,000g, 4°C). The supernatant is removed, quick-frozen on dry ice, and stored at -70°C. Protein concentrations are typically 5 to 10 mg/ml, and the telomerase activity is stable to multiple freeze-thaws.

Assay tubes are prepared by lyophilizing 0.1 µg of CX primer (5'-CCCTTACCCTTACCCTTACCCTAA-3') onto the bottom of the tube and sealing it with 7 to 10 µl of molten wax (Ampliwas, Perkin-Elmer). After the wax was allowed to solidify at room temperature, the tubes are stored at 4°C. Fifty-microliter TRAP reactions above the wax barrier contain 20 mM tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 63 mM KCl, 0.005% Tween-20, 1 mM EGTA, 50 µM deoxynucleoside triphophates, 0.1 µg of TS oligonucleotide (5'-AATCCGTCGAGCAGAGTT-3'), 1 µg of T4 gene 32 protein (Boehringer Mannheim), bovine serum albumin (0.1 mg/ml), 2 U of Taq DNA polymerase (Boehringer Mannheim), and 1 to 2 µl of a CHAPS cell extract. For radiolabeling of products, 0.2 to 0.4 µl of [α-^{32}P]dGTP (deoxyguanosine triphosphate) or [α-^{32}P]dCTP (deoxycytidine triphosphate) (10 µCi/µl, 3000 Ci/mmol) are added to the reaction. After 10 min at 23°C for extension of oligonucleotide TS by telomerase, tubes are transferred to a thermal cycler for 27 rounds at 94°C for 30 s, 50°C for 30 s, and 72°C for 1.5 min. The CX primer (0.1µg) is liberated when the wax barrier melted at about 70°C. One-half of the reaction is analyzed by electrophoresis in 0.5 x tris-borate EDTA on 15% polyacrylamide nondenaturing gels.

Pharmaceutical Compositions

A wide variety of conditions associated with inappropriate or undesired cell proliferation can be treated by administration of a composition comprising oligonucleotide N3'->P5' phosphoramidates of the invention. Such conditions include cancers associated with positive telomerase activity, such as ovarian cancers, neuroblastomas, and the like. Other cancers include leukemias, and adenocarcinomas, sarcomas, and epidermoid carcinomas of various organs, including prostate, lung, breast, liver, kidney, and brain. Preferably, cancers suseptible to treatment are identified and/or confirmed by testing for telomerase activity, e.g.

using an assay as described in U.S. patent 5,489,508. Additional conditions amenable to treatment by telomerase inhibition include infections from eukaryotic organisms, such as fungus and protozoan parasites. Exemplary fungi include Candida, including C. albicans, C. tropicalis, C. parapsilosis, C. guilliermondii, C. krusei, and the like; Aspergillus, including A. fumigatus, A. flavis, A. niger, and the like, Cryptococcus, Rhizopus, Mucor, and the like.

Pharmaceutical compositions of the invention can vary widely depending on the target tissue or cell types being treated, but generally consist of an oligonucleotide N3'->P5' phosphoramidate and a pharmaceutical carrier. Pharmaceutical carriers typically include inert non-toxic buffering agents. The carriers may also include agents to enhance or regulate delivery to target cells or tissues, e.g. dispersing agents, solubilizing agents for transdermal delivery, and the like. Typical inert carriers include sterile water, alcohol, fats, waxes, neutral lipids, cationic lipids, and the like. For topical administration, the compositions of the invention may be formulated into ointments, salves, gels, or creams, as generally known in the art. Generally, compositions useful for parenteral administration of drugs are well known, e.g. Remington's Pharmaceutical Science, 15th ED. (Mack Publishing Company, Easton, PA, 1980). Compositions of the invention may also be administered by way of an implantable or injectable drug delivery system, e.g. Urquhart et al, Ann. Rev. Pharmacol. Toxicol., Vol. 24, pgs. 199-236 (1984); Lewis, ed. Controlled Release of Pesticides and Pharmaceuticals (Plenum Press, New York, 1981); U.S. patent 3,773,919; U.S. patent 3,270,960; or the like. Compounds of the invention may also be conjugated to transport moieties to aid in targeting tissues or in penetrating cell membranes and the like, e.g. as taught by Latham et al, PCT application WO 91/14696.

Compositions of the invention may be administered or intravenously. In such cases, pharmaceutical carriers include saline solutions, dextrose solutions, combinations of the two, nonaqueous solutions such as ethyl oleate, and the like. Other delivery means include liposomes, other controlled release devices, iontophoresis, electroporation, and the like.

Selecting an administration regimen and route of administration for a composition of the invention depends on several factors, including the rate of degradation of the particular compounds in serum, the accessibility of the target tissues and cells, pharmacokinetics, toxicity, patient weight, patient age, type and state of disease, and the like. Preferably, an administration regimen maximizes the amount of compound delivered to a patient consistent with an acceptable level of side effects. Accordingly, the amount of compound delivered may depend on the particular compound, the severity of the infection or other condition being treated, and the like. Routes of administration include intramuscular, aerosol, oral, topical, systemic, and ocular. Guidance for selecting appropriate delivery means, routes of administration, and dosages is readily found in the medical literature.

For a given disease or condition, an effective amount of compound of the invention is administered. As used herein, "effective amount" means an amount sufficient to bring about therapeutically useful inhibition without significant side-effects. Clearly, if effective inhibition can be achieved with no side-effects with compounds of the invention at a certain concentration, then that concentration should be used as opposed to a higher concentration at which side-effects become evident. If side-effects are unavoidable, the minimum amount of compound that is necessary to achieve the inhibition desired should be used. Preferably, dosages comprising effective amounts of the compounds of the invention for systemic treatment are sufficient to provide a concentration at the target tissue of from about 40-50 nanomolar to about 20-50 micromolar. More preferably, this range is from 50 nanomolar to 20 micromolar. For topical or local treatments, dosages comprising effective amounts of compound are sufficient to provide a concentration at the target tissue of from about fifty to several hundred nanomolar to about 100-200 micromolar. More preferably, this range is from 100 nanomolar to 200 micromolar.

Example 1

5'-DMT-O-2'-fluoro-3'-aminouridine

Compound 1, 5'-DMT-2'-fluoro-3'-aminouridine was prepared according to Scheme 1 shown in Figure 1. This monomer was used for incorporation of 2'-fluoro-3'-aminouridine into oligonucleotide phosphoramidates by the oxidative phosphorylation method. First, uridine 3 was transformed into the 5'-DMT-2',3'-anhydrolyxouridine 4 by successive, one pot reaction with DMT-chloride, mesyl chloride, and sodium hydroxide. The 2',3'-epoxy ring was then opened by treatment with sodium azide (Webb et al, J. Med. Chem., 31: 1475-1479 (1988)), producing 5'-DMT-3'-azido-3'-deoxyarabinouridine 5 as the main product, and isomeric 5'-DMT-2'-azido-2'-deoxyxylouridine as a by-product, in approximately a 2:1 ratio. Compound 5 was isolated by silica gel chromatography and then fluorinated with diethylaminosulfur trifluoride (DAST) to give 6. Finally, the azido group of 6 was reduced with hydrogen over palladium catalyst, giving 5'-DMT-2'-fluoro-3'-aminouridine 1. The structure of nucleoside 1 was confirmed by ^{1}H and ^{19}F NMR analysis and by mass spectrometry (Figure 5). More particularly, the steps were carried out as follows:

5'-O-DMT-2',3'-anhydrolyxouridine (compound 4, Figure 1) was prepared as follows: To 10.0 g (41 mmol) of dry 3 in 250 mL anhydrous pyridine was added 14.6 g (43 mmol) of dimethoxytrityl chloride. The mixture was stirred overnight, then cooled on ice and 8.0 mL (103 mmol) methanesulfonyl chloride was added. After stirring at room temperature for 4 h, the reaction was quenched with 1 mL H_2O and the solvent removed *in vacuo*. The residue was suspended in 500 mL CH_2Cl_2, washed with water (3x250 mL), and

reconcentrated *in vacuo* to a foam. The foam was dissolved in 450 mL dioxane and 250 mL of 1 N NaOH added. After stirring for 4 h, the dioxane was removed *in vacuo*, and the resultant slurry extracted with 500 mL of CH_2Cl_2. Concentration of the CH_2Cl_2 layer and flash chromatography provided 13.8 g (64%) of product as a light yellow powder. Mass-spectrometry, FAB^+, $M+H^+$, calculated: 529.1975, observed: 529.1963. ^1H NMR δ 7.58 (d, J = 8.2 Hz, 1H), 7.5-7.2 (mm, 10H), 6.86 (d, J = 8.2 Hz, 4H), 6.20 (s, 1 H), 5.68 (d, J = 8.1 Hz, 1H), 4.20 (dd, J = 5.8, 5.8 Hz, 1H), 3.96 (d, J = 2.9 Hz, 1H), 3.92 (d, J = 3.0 Hz, 1H), 3.82 (s, 6H), 3.47 (dd, J = 5.9, 9.7 Hz, 1H), 3.38 (dd, J = 5.7, 9.6 Hz, 1H).

5'-O-DMT-3'-azido-3'-deoxyarabinouridine (compound 5, Figure 1) was prepared as follows: To 13.8 g (26 mmol) of 4 in 500 mL acetone was added 200 mL H_2O and 12.0 g (185 mmol) NaN_3. The mixture was refluxed overnight, then concentrated *in vacuo* to remove the acetone. The resultant slurry was extracted with 600 mL CH_2Cl_2, which was in turn was washed with water (3x250 mL). Concentration of the CH_2Cl_2 layer and flash chromatography of the crude product provided 6.0 g (40%) of a pale yellow solid. Mass-spectrometry, FAB^+, $M+H^+$, calculated: 572.2145, observed: 572.2147. ^1H NMR δ 9.4 (br s, 1H), 8.07 (d, J = 8.1 Hz, 1H), 7.4-7.3 (mm), 6.89 (d, J = 7.9 Hz, 4H), 6.10 (d, J = 5.5 Hz, 1H), 5.46 (d, J = 8.1 Hz, 1H), 4.55 (m, 1H), 4.19 (dd, J = 7.2, 7.7 Hz, 1H), 3.84 (m, 1H), 3.83 (s, 6H), 3.64 (dd J = 2.6, 11.3 Hz, 1H), 3.43 (dd, J = 2.6, 11.4 Hz, 1H).

5'-O-DMT-2'-fluoro-3'-azido-2',3'-dideoxyuridine (compound 6, Figure 1) was prepared as follows: To 6.0 g (10.5 mmol) of 5 in 120 mL anhydrous DMF was added 2.4 mL (18.2 mmol) of diethylaminosulfur trifluoride. The mixture was stirred for 16 h, then poured into 300 mL of cold saturated aqueous $NaHCO_3$. The product was extracted with 500 mL ethyl acetate, which in turn was washed with water (2x500 mL). Concentration of the organic layer and flash chromatography of the crude product provided 2.9 g (48%) of an off-white solid. Mass-spectrometry, FAB^+, M^+, calculated: 573.2024, observed: 573.2011. ^1H NMR δ 8.95 (br s, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.2-7.4 (mm, 11H), 6.88 (d, J = 8.6 Hz, 4H), 6.02 (d, J = 17.2 Hz, 1H), 5.42 (d, J = 8.1 Hz, 1H), 5.27 (dd, J = 3.7, 55.0 Hz, 1H), 4.24 (m, 1H), ~4.21 (partially overlapping with signal 4.24 ppm, presumed ddd, J = 4, 4, ~25 Hz, 1H), 3.82 (s, 6H), 3.71 (d, J = 11.4 Hz, 1H), 3.49 (d, J = 11.3 Hz, 1H); ^{19}F NMR δ -196.7 (dddd, est. J = 3, 17, 25, 55 Hz).

5'-O-DMT-2'-fluoro-3'-amino-2',3'-dideoxyuridine (compound 6, Figure 1) was prepared as follows: To 2.9 g (5.1 mmol) of 6 in 75 mL of 95% ethanol was added 0.5 g of 10% palladium on carbon. The mixture was hydrogenated at 40 psi overnight and then the catalyst removed by filtration. The solvent was removed *in vacuo*, and the resultant solid purified by flash chromatography to give 1.2 g (43%) of product as a white powder. Mass-spectrometry, FAB^+, $M+H^+$, calculated: 548.2197, observed: 548.2206. ^1H NMR δ 8.04 (d, J = 8.0 Hz, 1H), 7.9 (br m, 1H), 7.2-7.4 (mm), 6.85 (d, J = 8.4 Hz, 4H), 6.00 (d, J = 16.2

Hz, 1H), 5.32 (d, J = 8.7 Hz, 1H), 4.83 (dd, J = 3.9, 52.2 Hz, 1H), 3.88 (br d, J = 11 Hz, 1H), 3.8 (s, 6H), 3.8-3.7 (mm, 2H), 3.52 (dd, J = 2.6, 11.1 Hz, 1H); ^{19}F NMR δ -200.1 (ddd, J = 16.4, 27.5, 52.1 Hz).

Example 2

N^4-benzoyl-2'-fluoro-3'-(4-methoxytrityl)amino-2',3'-dideoxyuridine 5'-(2-cyanoethyl N,N-diisopropyl)phosphoramidite

Oligomers of the invention were also synthesized from phosphoramidite monomer, as illustrated by compound **2u** of Scheme 2. Uridine **3** was mesylated and then selectively benzoylated with accompanying formation of the 2,2'-anhydrocycle by treatment with sodium benzoate according to Codington et al, J. Am. Chem. Soc., 82: 2794-2803 (1960)). These reactions resulted in compound **7** with 69-77% overall yields. 2,3'-anhydroarabinonucleoside **7** was transformed into 2',3'-anhydrolyxouridine **8** in two steps by the method described in Codington et al, J. Organic Chem. 27: 163-167 (1962). This involved treatment of **7** with hydrochloric acid to form 3'-mesyl-5'-benzoylarabinouridine, which upon treatment with ammonium hydroxide closed to form the lyxo-2',3'-epoxide **8** in 63-77% overall yields. 2',3'-anhydrolyxonucleoside **8** was then heated with ammonium azide, in a reaction described by Reichman et al, J. Organic Chem., 41: 2042-2043 (1976). Contrary to literature suggestion, this reaction was not completely stereoselective, but produced a chromatographically unresolvable mixture of the desired 5'-benzoyl-3'-azidoarabinonucleoside **9** and it's 2'-azido-2'-deoxyregioisomer **9i** in approximately a 2.5:1 ratio. Crude arabinonucleoside **9** was fluorinated with DAST to give 2'-fluoro-3'-azidonucleoside **10**, then catalytically hydrogenated to give 2'-fluoro-3'-aminonucleoside **11**, which was separable from its regioisomer by silica gel chromatography. Protection of the 3'-amine with a monomethoxytrityl (MMT) group, followed by 5'- debenzoylation produced intermediate **13**, with 5'-phosphitylation producing the desired phosphoramidite building block **2u** in a 22% overall yield from anhydronucleoside **8**. More particularly, the steps were carried out as follows:

3'-O-Methanesulfonyl-5'-O-benzoyl-2,2'-anhydroarabinouridine (compound **7**, Figure 2) was prepared in two steps from **3** (Figure 2) according to the procedure of Codington et al (JACS, cited above) in 69-77% overall yields.

5'-O-benzoyl-2',3'-anhydrolyxouridine (compound **8**, Figure 2) was prepared in two steps from **7** according to the procedure of Codington et al (JOC cited above) in 63-77% overall yields.

3'-azido-5'-O-benzoyl-3'-deoxyarabinouridine (compound **9**, Figure 2) was prepared from **8** and anhydrous NH_4N_3, according to the procedure of Reichman et al (cited above) but without successful recrystallization. Mass yields were 98% or greater, but NMR

suggested 25-35% of the regioisomer, 2'-azido-5'-O-benzoyl-2'-deoxyxylouridine, **9i**, which coeluted with the desired product by silica gel TLC. ^{1}H NMR, Major component, **9**: δ 10.8 (br s, 1H), 8.11 (d, J = 7.5 Hz, 2H), 7.68 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 7.3 Hz, 1H), 7.5 (m, 2H), 6.19 (d, J = 3.6 Hz, 1H), 5.40, (d, J = 8.0 Hz, 1H), 4.84 (m, 1H), 4.73 (d, J = 5.7 Hz, 1H), 4.63 (br d, J = 4.2 Hz, 1H), 4.2 (mm, 2H); Minor component, **9i**: δ 10.6 (br s, 1H), 8.11 (d, J = 7.5 Hz, 2H), 7.81 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 7.5 Hz, 1H), 7.5 (m, 2H), 5.85 (s, 1H), 5.47, (d, J = 8.1 Hz, 1H), 4.86 (m, 1H), 4.76 (d, J = 5.4 Hz, 1H), 4.62 (br d, J = 4.0 Hz, 1H), 4.3-4.2 (mm, 2H).

2'-fluoro-3'-azido-5'-O-benzoyl-2',3'-dideoxyuridine (compound 10, Figure 2) was prepared as follows: To 5.0 g (13.4 mmol) of crude **9** (containing 25% **9i**) in 30 mL anhydrous CH_2Cl_2 was added 8.8 mL (66.6 mmol) of diethylaminosulfur trifluoride. After stirring for 48 h, the mixture was diluted with 100 mL CH_2Cl_2 and poured into 200 mL saturated aqueous $NaHCO_3$. When evolution of gas ceased, the CH_2Cl_2 layer was washed with 100 mL fresh $NaHCO_3$ solution and then with water (2x100 mL). Concentration of the CH_2Cl_2 layer *in vacuo* and flash chromatography gave 3.5 g (70%) of product containing 20% of the largely chromatographically unresolvable isomeric impurity, **10i**. ^{1}H NMR, Major component, **10**: δ 8.7 (br s, 1H), 8.07 (d, J = 7.4 Hz, 2H), 7.62 (d, J = 7.5 Hz, 1H), 7.49 (dd, J = 7.6, 7.6 Hz, 2H), 7.39 (d, J = 8.1 Hz, 1H), 5.70 (d, J = 21.1 Hz, 1H), 5.65 (d, J = 8.2 Hz, 1H), 5.48 (dd, J = 4.7, 52.9 Hz, 1H), 4.7-4.4 (unresolved), 4.32 (dd, J = 4.7, 9.5 Hz, 1H), 4.27 (dd, J = 4.7, 9.5 Hz, 1H); Minor component, **10i**: δ 8.7 (br s, 1H), 8.03 (d, J = 7.2 Hz, 2H), 7.64 (d, J = 7.6 Hz, 1H), 7.51 (dd, J = 7.4, 7.7 Hz, 2H), 7.33 (d, J = 8.2 Hz, 1H), 5.99 (d, J = 6.4 Hz, 1H), 5.67 (d, J = 9 Hz, 1H), 5.40 (ddd, J = 2.8, 5.0, 53.4 Hz, 1H), 4.8-4.4 (unresolved), 4.10 (mm, 2H).

2'-fluoro-3'-amino-5'-O-benzoyl-2',3'-dideoxyuridine (compound 11, Figure 2) was prepared as follows: To 3.5 g (9.3 mmol) crude **10** (20% **10i**) in 200 mL 95% ethanol was added 600 mg of 10% palladium on carbon. The suspension was hydrogenated at 40 psi overnight and then the catalyst removed by filtration. The solvent was removed *in vacuo*, giving 2.93 g (90%) of a light yellow solid consisting of two compounds which were resolvable by TLC. Flash chromatography provided 1.96 g (60% yield) of the desired product as a pure white solid. Mass-spectrometry, FAB^+, $M+H^+$, calculated: 350.1152, observed: 350.1152. ^{1}H NMR δ 8.14 (br s, 1H), 8.06 (d, J = 7.1 Hz, 1H), 7.64 (dd, J = 7.4, 7.4 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.50 (dd 7.7, 7.8 Hz, 1H), 5.86 (d, J = 18.5 Hz, 1H), 5.51 (d, J = 8.2 Hz, 1H) 5.00 (dd, J = 4.3, 52.4 Hz, 1H), 4.81 (dd J = 2.2, 12.8 Hz, 1H), 4.73 (dd, J = 3.5, 12.7 Hz, 1H), 4.14 (ddd, J = 2, 3, 10.2 Hz, 1H), 3.57 (ddd, J = 4, 10.5, 26.6 Hz, 1H); ^{19}F NMR δ -198.3 (ddd, J = 18.5, 26.4, 52.2 Hz).

2'-fluoro-3'-(4-methoxytrityl)amino-2',3'-dideoxyuridine (compound 12, Figure 2) was prepared as follows: To 1.0 g (2.9 mmol) of **11** in 50 mL anhydrous pyridine was added

1.0 g (3.2 mmol) 4-methoxytrityl chloride. The mixture was stirred overnight, 5 mL saturated aqueous $NaHCO_3$ was added, and the mixture concentrated *in vacuo* to an oil. The oil was dissolved in 125 mL ethyl acetate, which was washed with water (3x100 mL) and reconcentrated *in vacuo* to 2.05 g of foam.

The foam was dissolved in a mixture of 40 mL methanol, 40 mL dioxane, and 10 mL water. NaOH (1 g, 25 mmol) was added and the mixture stirred overnight. The solution was concentrated *in vacuo* to a syrup, which was dissolved in 100 mL ethyl acetate and washed with water (3x100mL). Concentration *in vacuo* of the organic layer gave 1.11 g of a foam, which upon flash chromatography gave 1.05 g (76%) of a white solid. Mass-spectrometry, FAB^+, $M+H^+$, calculated: 518.2091, observed: 518.2076. ^1H NMR δ 8.64 (br d J = 4.2 Hz, 1H), 8.14 (br s, 1H), 7.57 (mm, 5H), 7.48 (d J = 8.7 Hz, 1H), 7.3 (mm, 8H), 6.83 (d J = 8.8 Hz, 2H), 5.67 (d, J = 17.7 Hz, 1H), 5.62 (d, J = 8.1 Hz, 1H), 4.23 (m, 2H), 4.03 (br d, J = 10.2 Hz, 1H), 3.80 (s, 3H), 3.31 (dddd, J = 3.6, 10.3, 10.9, 25.8 Hz, 1H), 2.80 (dd, J = 3.6, 50.9 Hz, 1H), 2.51 (dd, J = 3.0, 11.2 Hz, 1H); ^{19}F NMR δ -192.5 (dddd, J = 2.9, 17.7, 26.1, 50.9 Hz).

N^4-benzoyl-2'-fluoro-3'-(4-methoxytrityl)amino-2',3'-dideoxyuridine 5'-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (compound 2u, Figure 2) was prepared as follows: To 700 mg (1.35 mmol) of **12** in 20 mL anhydrous CH_2Cl_2 was added 200 mg (1.17 mmol) of diisopropylammonium tetrazolide and 0.5 mL (1.57 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite. After stirring the mixture for 3h, the solvent was removed *in vacuo* and the residue purified on a Chromatotron, using 4 mm plates and eluting with 0-3% methanol, 0.5% triethylamine in CH_2Cl_2. The product was concentrated *in vacuo* to an oil, which was dissolved in 10 mL CH_2Cl_2 and precipitated by slow addition into 100 mL of rapidly stirred hexane. After decanting the supernatant, the product was vacuum desiccated over P_2O_5, giving 680 mg (70%) of white powder. Mass-spectrometry, FAB^+, $M+H^+$, calculated: 718.3170, observed: 718.3194. ^{19}F NMR δ -190.9 (ddd, J = 21.7, 21.8, 51.3 Hz); ^{31}P NMR δ 150.5, 149.5.

Example 3

N^4-benzoyl-2'-fluoro-3'-(4-methoxytrityl)amino-2',3'-dideoxycytidine 5'-(2-cyanoethyl N,N-diisopropyl)phosphoramidite

Crude intermediate **10** was used for preparation of the appropriately protected cytidine phosphoramidite **2c** (Scheme 3, Figure 3). The uracil base of **10** was converted to cytosine by adaptation of the method of Divakar et al, J. Chem. soc., Perkin. Trans. 1 1982: 1171-1176. Subsequent 4-N benzoylation and reduction of the 3'-azido to an amino group gave compound **13**, which was separable from its regioisomer by silica gel chromatography. Protection of the 3'-amine with an MMT group, followed by selective 5'-O-debenzoylation

produced intermediate **15**. Subsequent 5'-phosphitylation lead to desired phosphoramidite **2c** in a 10% overall yield based on anhydronucleoside **8**. More particularly, the steps were carried out as follows:

N^4,5'-O-dibenzoyl-2'-fluoro-3'-amino-2',3'-dideoxycytidine (compound **13**, Figure 3) was prepared as follows: To 6.9 g (18.4 mmol) of crude **10** (containing 35% **10i**) in 50 mL anhydrous CH_3CN was added an ice-cold solution of 11.7 g (169 mmol) 1,2,4-triazole and 3.35 mL (36.1 mmol) $POCl_3$ in 90 mL anhydrous CH_3CN. The mixture was cooled in an ice bath and anhydrous triethylamine (23 mL, 165 mmol) was added, then the reaction allowed to warm to room temperature with stirring. After 90 min, 15 mL (108 mmol) triethylamine and 4 mL water were added and the mixture stirred for 10 min. The solvent was removed *in vacuo*, then 250 mL ethyl acetate was added, and the solution was washed with water. TLC indicated a fluorescent intermediate with the same mobility as the starting material.

The mixture was concentrated *in vacuo* to 6.7 g of a foam. Dioxane (100 mL) and 20 mL concentrated aqueous ammonia were added, and after 3 h, the mixture was concentrated *in vacuo* to a yellow gel. The gel was dissolved in 100 mL ethyl acetate and washed with water (3x200 mL). Concentration *in vacuo* and vacuum desiccation over P_2O_5 yielded 5.4 g of a solid which gave only one spot by silica gel TLC. Only two significant signals were observed by ^{19}F NMR, Major component: δ -192.8 (ddd, J = 22.8, 22.8, 53.1 Hz); Minor component: δ -200.7 (ddd, J = 13.6, 19.9, 53.4 Hz).

Anhydrous pyridine (100 mL) was added and the solution cooled to 4 °C. Benzoyl chloride (11.7 mL 100 mmol) was added with stirring and the mixture allowed to warm to room temperature. After 2 h, 5 mL water was added and the solvent removed *in vacuo*, giving a brown oil, which was dissolved in 200 mL ethyl acetate, washed with water (3x200 mL), and then reconcentrated *in vacuo* to an oily foam.

Ethanol (150 mL) and 2 g of 10% palladium on activated carbon were added and the mixture was hydrogenated at 40 psi H_2 overnight. TLC indicated formation of two new slower, closely-migrating compounds.

The catalyst was removed by filtration, and the filtrate concentrated *in vacuo* to an oily yellow foam. Silica gel flash chromatography (500 mL silica, eluted with 0-3% CH_3OH in CH_2Cl_2) provided 1.85 g of semi-pure product, which was dissolved in 10 mL CH_2Cl_2. A solid quickly precipitated, which was collected by filtration and washed with fresh CH_2Cl_2. Vacuum desiccation yielded 1.5 g of product **13** (11% yield from **9** and **9i**) as fine white crystals. Mass-spectrometry, FAB^+, $M+H^+$, calculated: 453.1574, observed: 453.1574. ^1H NMR δ 8.21 (d, J = 7.5 Hz, 1H), 8.08-8.13 (mm, 3H), 7.94 (d, J = 7.4 Hz, 2H), 7.46-7.7 (mm, 8H), 6.04 (d, J = 16.9 Hz, 1H), 5.08 (dd, J = 3.6, 51.5 Hz, 1H), 4.85 (dd, J = 3.3, 12.8 Hz, 1H), 4.80 (dd, J = 2.1, 12.8 Hz, 1H), 4.26 (m, 1H), 3.48 (dm, J = 27 Hz, 1H); ^{19}F NMR δ -200.1 (m).

N^4,5'-O-dibenzoyl-2'-fluoro-3'-(4-methoxytrityl)amino-2',3'-dideoxycytidine (compound 14, Figure 3) was prepared as follows: To 0.9 g (2.0 mmol) of **13** in 25 mL anhydrous pyridine was added 0.86 g (2.8 mmol) 4-methoxytrityl chloride, and the mixture stirred overnight. The reaction was quenched with 0.5 mL H_2O and concentrated *in vacuo*. CH_2Cl_2 (50 mL) was added and washed with 50 mL saturated aqueous $NaHCO_3$ and with water (2x50 mL). The solvent was removed *in vacuo*, replaced with 10 mL CH_2Cl_2, and pipetted into 80 mL rapidly stirred 1/1 hexane/ether. After further stirring for 2 h, the product was collected by filtration and dried under vacuum, giving 1.3 g (88% yield) of product as a white powder. Mass-spectrometry, FAB^+, $M+H^+$, calculated: 725.2775, observed: 725.2761. ^1H NMR δ 8.59 (br s, 1H), 8.07 (br d, J = 5.7 Hz, 1H), 7.89 (br d, J = 7 Hz, 2H), 7.83 (dd, J = 1.3, 6.7 Hz, 2H), 7.68 (dd, J = 7.4, 7.4 Hz, 2H), 7.5-7.6 (m, 8H), 7.43 (dd, J = 2.1, 6.9 Hz, 2H), 7.1-7.3 (mm, 7H), 6.71 (d, J = 8.9 Hz, 2H), 5.80 (d, J = 15.4 Hz, 1H), 5.03 (dd, J = 2.0, 13.0 Hz, 1H), 4.98 (dd, J = 2.3;, 13.1 Hz, 1H), 4.41 (br d, J = 10.5 Hz, 1H), 3.63 (s, 3H), 3.36 (dddd, J = 3.1, 11.1, 11.1, 25.7 Hz, 1H), 2.84 (dd, J = 3.1, 49.9 Hz, 1H), 2.52 (dd, J = 2.7, 11.5 Hz, 1H); ^{19}F NMR δ -196.3 (m).

N^4-benzoyl-2'-fluoro-3'-(4-methoxytrityl)amino-2',3'-dideoxycytidine (compound 15, Figure 3) was prepared as follows: To 1.3 g (1.75 mmol) of **14** in 20 mL of 65/30/5 pyridine/methanol/water, cooled in an ice bath, was added 10 mL of cold 2 M NaOH in 65/30/5 pyridine/methanol/water. The mixture was stirred cold for 20 min, then neutralized with pyridinium-H^+ form Bio-Rad AG® 50W-X8 cation exchange resin. After 5 min, the resin was removed by filtration and washed with methanol. The combined filtrate and wash were concentrated *in vacuo* to an oil, which was dissolved in 100 mL ethyl acetate. The mixture was washed with 100 mL saturated aqueous $NaHCO_3$ and with water (2x100mL). After concentration *in vacuo* to a foam, the product was dissolved in 10 mL CH_2Cl_2 and pipetted into 75 mL rapidly stirred hexane/ether, 2/1. The product was collected by filtration and dried under vacuum, giving 1.13 g (102% yield) of product as a white powder. Mass-spectrometry, FAB^+, $M+Cs^+$, calculated: 753.1489, observed: 753.1499. ^1H NMR δ 8.30 (br d, J = 6.8 Hz, 1H), 7.89 (br d, J = 6.7 Hz, 2H), 7.64 (dd, J = 7.4, 7.4 Hz, 1H), 7.44-7.56 (mm, 9H), 7.22-7.32 (mm, 9H), 6.82 (d, J = 8.8 Hz, 2H), 5.80 (d, J = 15.7 Hz, 1H), 4.26 (mm, 2H), 4.13 (d, J = 10.2 Hz, 1H), 3.81 (s, 3H), 3.26 (dddd, J = 3.4, 10.7, 10.8, 26.5 Hz, 1H), 2.93 (dd, J = 3.3, 50.5 Hz, 1H), 2.50 (dd, J = 2.8, 11.0 Hz, 1H); ^{19}F NMR δ -195.3 (m).

N^4-benzoyl-2'-fluoro-3'-(4-methoxytrityl)amino-2',3'-dideoxycytidine 5'-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (compound 2c, Figure 3) was prepared as follows: To 970 mg (1.56 mmol) of **15** in 25 mL anhydrous CH_2Cl_2 was added 200 mg (1.17 mmol) of diisopropylammonium tetrazolide and 1.0 mL (3.15 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite. After stirring the mixture for 3h, the solvent was

removed *in vacuo* and the residue purified on a Chromatotron, using 4 mm plates and eluting with 0-1.5% methanol in 0.5% triethylamine in CH_2Cl_2. The product was concentrated *in vacuo* to a foam, which was dissolved in 10 mL CH_2Cl_2, and precipitated by slow addition to 40 mL of rapidly stirred hexane. After decanting the supernatant, the product was vacuum desiccated over P_2O_5, giving 880 mg (69%) of white powder. Mass-spectrometry, FAB^+, $M+Cs^+$, calculated: 953.2568, observed: 953.2531. ^{19}F NMR δ -193.6 (m); ^{31}P NMR δ 150.4, 149.4.

Example 4

N⁴-benzoyl-2'-fluoro-3'-(4-methoxytrityl)amino-2',3'-dideoxycytidine 5'-succinyl-loaded CPG

Intermediate 15 was 5'-succinylated and loaded upon CPG solid support by standard procedures, e.g. Knorr et al, Tetrahedron Lett. 30: 1927-1930 (1989) and Atkinson et al, pages 35-81, in Oligonucleotide Synthesis: A Practical Approach, Gait, editor (IRL Press, Washington, D.C.) More, particularly, N^4-benzoyl-2'-fluoro-3'-(4-methoxytrityl)amino-2',3'-dideoxycytidine 5'-succinyl-loaded CPG was prepared as follows: To 100 mg (0.16 mmol) of 15 in 2 mL anhydrous CH_2Cl_2 was added 55 mg (0.55 mmol) of succinic anhydride and 65 mg (0.53 mmol) of dimethylaminopyridine. The mixture was stirred for 2 h, the evaporated *in vacuo* to an oil. The oil was dissolved in 20 mL CH_2Cl_2, washed with 20 mL of saturated aqueous $NaHCO_3$ and with water (2x20mL), and then reconcentrated *in vacuo* to a foam. To the foam was added 1 mL 0.4 M diisopropylethylamine in DMSO/N-methylpyrrolidine, 1/1, and 0.7 mL 0.2 M 1-hydroxybenzotriazole, 0.2 M 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate in DMSO/N-methylpyrrolidine, 1/1. After 3 min, the mixture was drawn into a 10 mL syringe containing 1.2 g of long-chain alkylamino-CPG. An additional 5 mL DMSO wash was also drawn into the syringe. The CPG-nucleoside mixture was mixed for 1.5 h, then the CPG washed with 5 volumes of anhydrous acetonitrile. Unreacted CPG amino groups were acetylated by standard ABI capping solutions for 2 min. The CPG was again washed with 5 volumes of acetonitrile and 5 volumes of CH_2Cl_2. Nucleoside loading was determined to be approximately 5 μmole/g by standard trityl assay.

Example 5

Solid Phase Synthesis of Oligo-2'-fluoronucleotide N3'->P5' Phosphoramidates

Oligo-2'-fluoronucleotide N3'->P5' phosphoramidates were synthesized on solid phase supports using either 5'-DMT-2'-fluoro-3'-aminonucleoside monomers (Scheme 1) or phosphoramidite monomers (of Schemes 2 and 3).

Dimer $dU^{f}_{np}T$ was prepared using monomer of Scheme 1 via carbon tetrachloride-driven oxidative phosphorylation of dU^{f}_{n} by a 5'-H-phosphonate of the anchored dT. Product was analyzed and isolated by RP HPLC in 70% yield and the structure was confirmed by mass spectrometry and by acid catalyzed hydrolysis, which gave 2'-fluoro-3'-amino-2'-deoxyuridine and 5'-thymidylic acid. The same synthetic strategy was also used to introduce one or two 2'-fluoro-3'-aminonucleosides into longer oligonucleotide phosphoramidate chains. Compounds **20** and **21**, Table 1, containing one or two 2'-fluoronucleosides in the middle of the chain were prepared and isolated by IE HPLC. Coupling yields of the 2'-flouronucleoside **1** did not exceed 70-75% (in contrast to 94-96% for the 2'-deoxynucleosides), as judged by step-wise measurement of released DMT-cation and by IE HPLC analysis.

Example 6

Inhibition of Human Telomerase Activity

The following oligo-2'-deoxynucleotide N3'->P5' phosphoramidate compounds were synthesized and tested in human telomerase inhibition assays at concentrations in the range of from 1 to 30 nM: 5'-GGGCTTCTTCCT, 5'-CTTCCT, 5'-TTAGGG, 5'-TTAGGGTTAGGG, 5'-GGGTTAGGGTT, and 5'-GTTAGGGTTAG. Oligomer 5'-GTTAGGGTTAG displayed significant inhibitory activity.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lynx Therapeutics, Inc.

(ii) TITLE OF INVENTION: Telomerase Inhibitors

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Vincent M. Powers, Dehlinger & Associates
(B) STREET: Post Office Box 60850
(C) CITY: Palo Alto
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94306-0850

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch diskette
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Vers. #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Vincent M. Powers
(B) REGISTRATION NUMBER: 36,246
(C) REFERENCE/DOCKET NUMBER: 5525-0023.41/LYNX-037

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415-324-0880
(B) TELEFAX: 415-324-0960

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGGCTTCTTC CT 12

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 nucleotides
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TTAGGGTTAG GG 12

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGGTTAGGGT T 11

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTTAGGGTTA G 11

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCCTTACCCT TACCCTTACC CTAA 24

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AATCCGTCGA GCAGAGTT 18

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GTTAGGGTTA 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

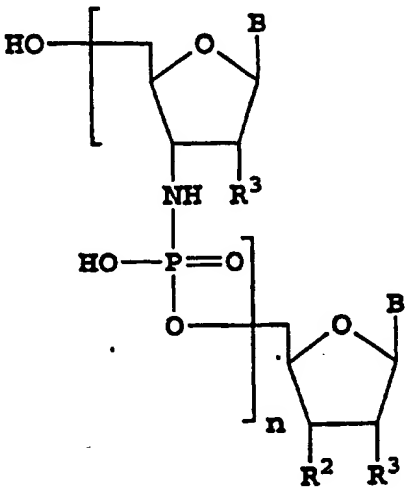
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TTAGGGTTAG 10

I claim:

1. A method of treating a condition associated with an elevated level of telomerase activity within a cell comprising the step of administering to the cell of an oligonucleotide N3'->P5' phosphoramidate compound having a sequence complementary to a portion of a telomere binding region of an RNA moiety of a telomerase of the cell, wherein said amount is effective to inhibit the activity of the telomerase in the cell.

2. The method of claim 1 wherein said oligonucleotide N3'->P5' phosphoramidate is defined by the formula:



wherein:

B is a purine, pyrimidine, or an analog thereof;

R^3 is hydrogen or fluoro;

R^2 is hydroxyl or amino; and

n is between 5 and 19.

3. The method of claim 2 or claim 3 wherein said telomerase is human telomerase.

4. The method of any of claims 1 to 3 wherein said portion of said telomere binding region is from 4 to 11 contiguous nucleotides.

5. The method of any of claims 1 to 4 wherein said oligonucleotide N3'->P5' phosphoramidate compound has a sequence selected from the group consisting of 5'-GTTAGGGTTAG, 5'- GTTAGGGTTA, 5'- TTAGGGTTAG, 5'- GTTAGGGTT, 5'-TTAGGGTTA, 5'- TAGGGTTAG, 5'- GTTAGGGT, 5'- TTAGGGTT, 5'-TAGGGTTA, 5'- AGGGTTAG, 5'- GTTAGGG, 5'- TTAGGGT, 5'- TAGGGTT, 5'-

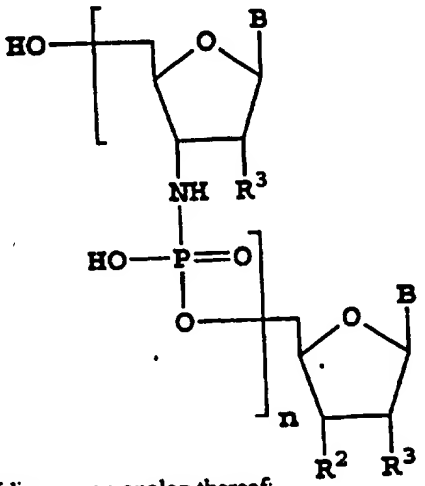
AGGGTTA, 5'- GGGTTAG, 5'- GTTAGG, 5'- TTAGGG, 5'- TAGGGT, 5'- AGGGTT, 5'- GGGTTA, and 5'- GGTTAG.

6. The method of any of claims 1 to 5 wherein said cell is a human cancer cell.

7. The method of any of claims 1 to 6 wherein said telomerase is a fungal telomerase selected from the group consisting of Candida albicans, Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Aspergillus fumigatus, Aspergillus flavis, and Aspergillus niger.

8. A pharmaceutical composition for inhibiting telomerase activity comprising a pharmaceutical carrier and an oligonucleotide N3'->P5' phosphoramidate having a sequence complementary to an RNA moiety of a telomerase.

9. The pharmaceutical composition of claim 8 wherein said oligonucleotide N3'->P5' phosphoramidate is defined by the formula:



wherein:

B is a purine, pyrimidine, or an analog thereof;

R^3 is hydrogen or fluoro;

R^2 is hydroxyl or amino; and

n is between 5 and 19.

10. The pharmaceutical composition of claim 8 or 9 wherein said telomerase is human telomerase.

11. The pharmaceutical composition of any of claims 8 to 10 wherein said oligonucleotide N3'->P5' phosphoramidate compound has a sequence selected from the group consisting of 5'-GTTAGGGTTAG, 5'- GTTAGGGTTA, 5'- TTAGGGTTAG, 5'-GTTAGGGTT, 5'- TTAGGGTTA, 5'- TAGGGTTAG, 5'- GTTAGGGT, 5'-TTAGGGTT, 5'- TAGGGTTA, 5'- AGGGTTAG, 5'- GTTAGGG, 5'- TTAGGGT, 5'-TAGGGTT, 5'- AGGGTTA, 5'- GGGTTAG, 5'- GTTAGG, 5'- TTAGGG, 5'-TAGGGT, 5'- AGGGTT, 5'- GGGTTA, and 5'- GGTTAG.

12. The pharmaceutical composition of claim 11 wherein said sequence of said oligonucleotide N3'->P5' phosphoramidate compound is 5'-GTTAGGGTTAG.

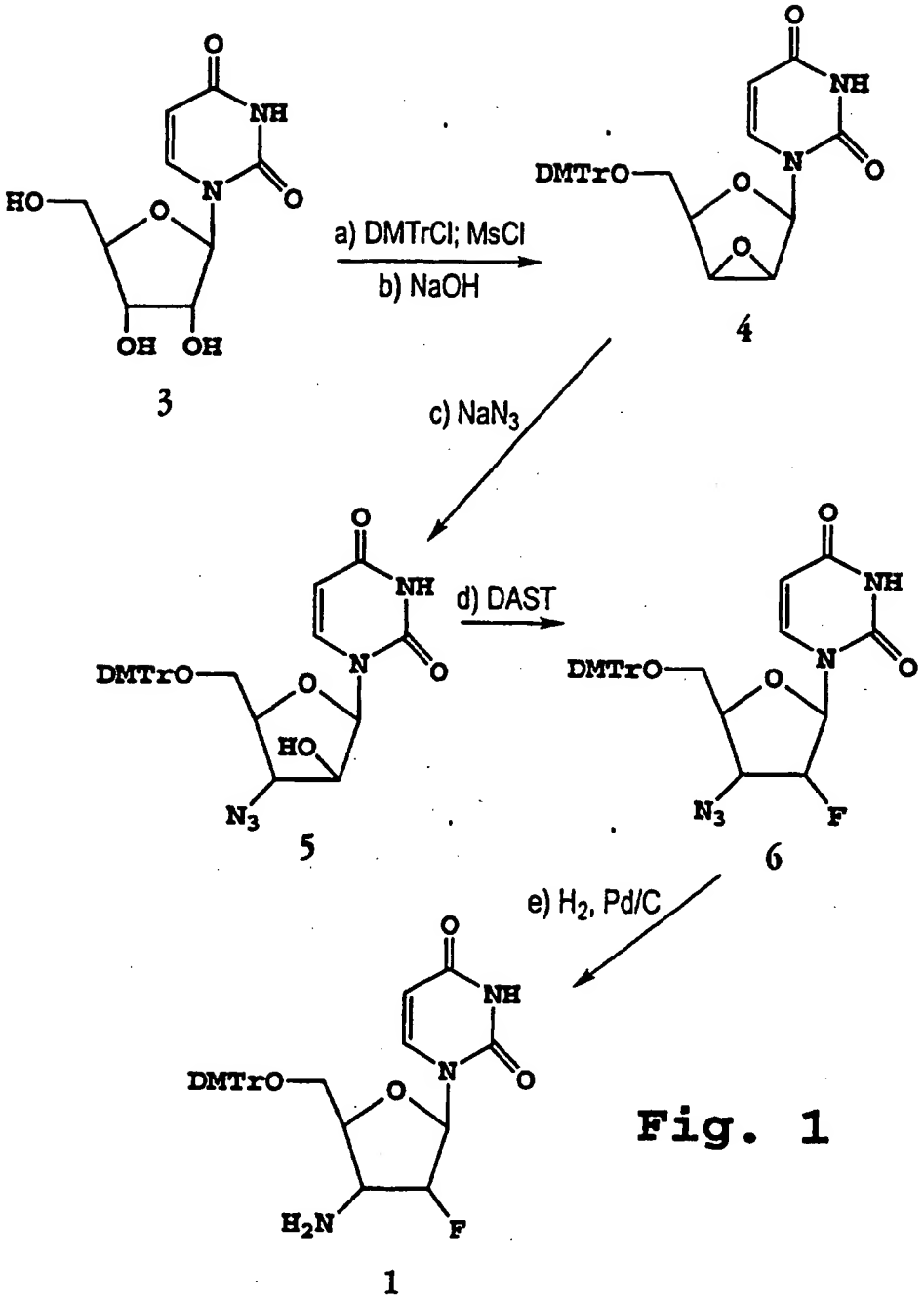

HO
NH
N
O
OH OH
3
a) DMTrCl; MsCl
b) NaOH
DMTrO
4
c) NaN_3
d) DAST
HO
N_3
5
F
6
e) H_2, Pd/C
H_2N
1

Fig. 1

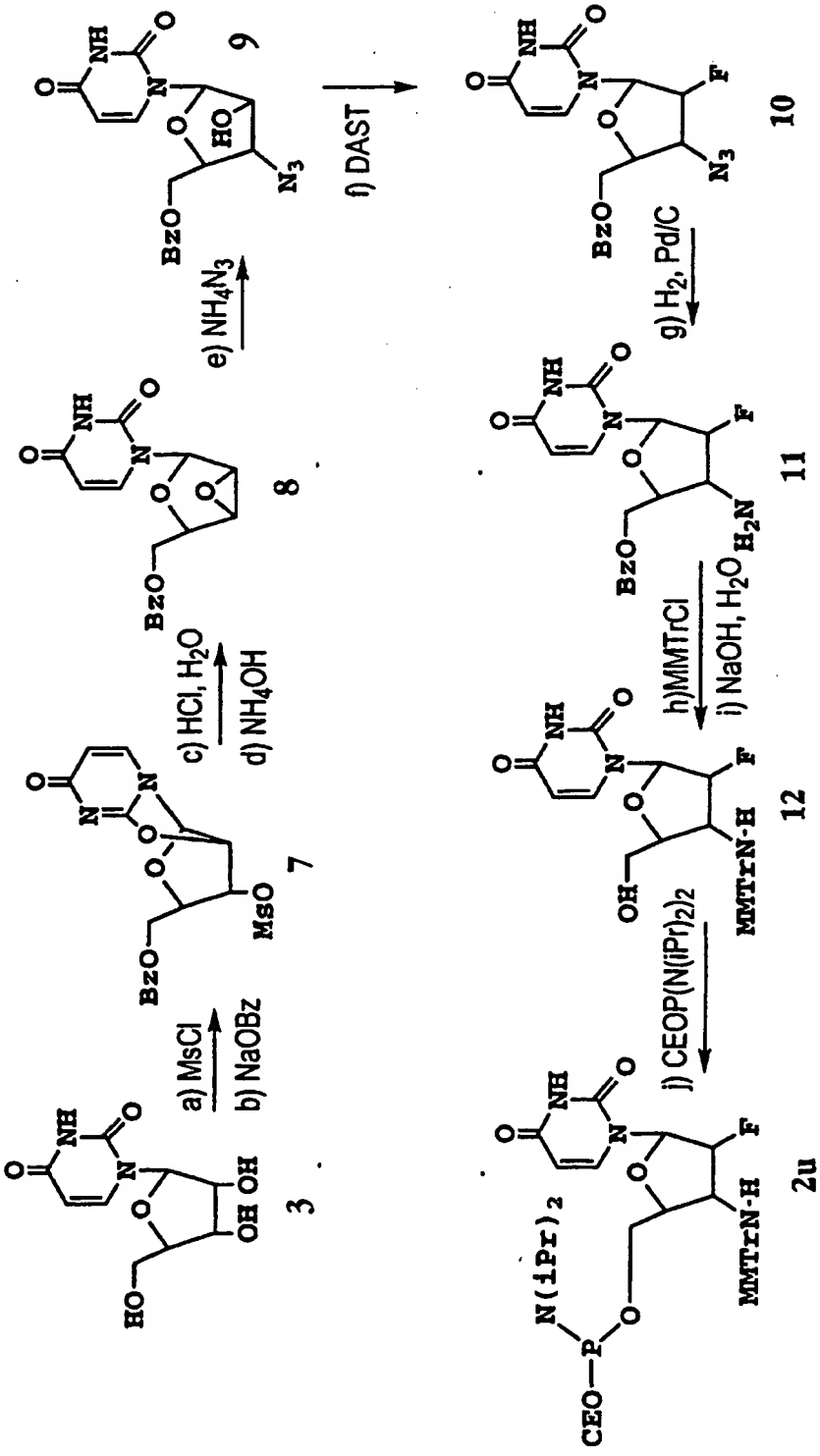

a) MsCl
b) NaOBz
c) HCl, H2O
d) NH4OH
e) NH4N3
f) DAST
g) H2, Pd/C
h)MMTrCl
i) NaOH, H2O
j) CEOP(N(iPr)2)2
3
7
8
9
10
11
12
2u

Fig. 2

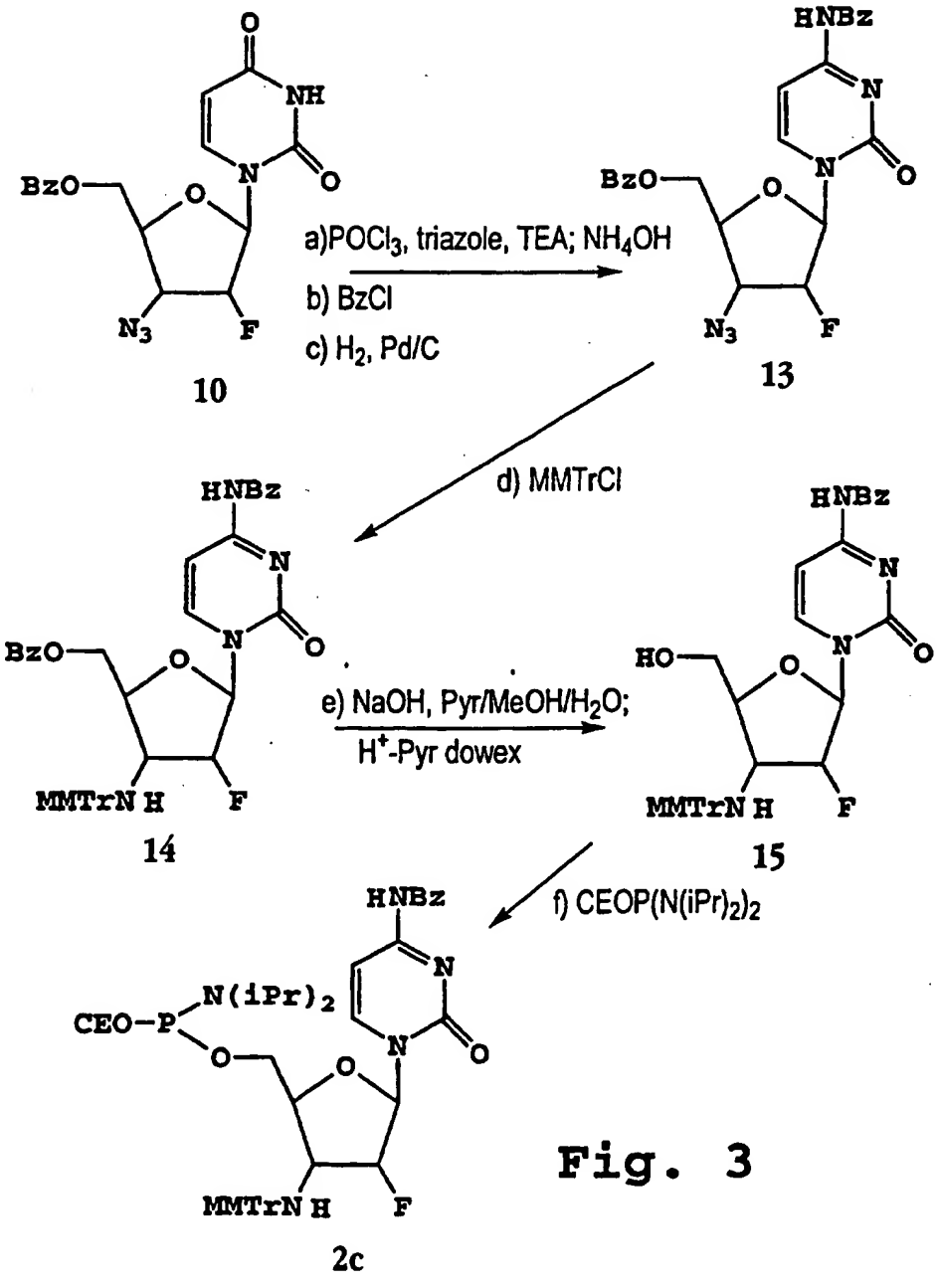

BzO
NH
N
O
O
N3
F
10
a)POCl3, triazole, TEA; NH4OH
b) BzCl
c) H2, Pd/C
HNBz
13
d) MMTrCl
14
e) NaOH, Pyr/MeOH/H2O;
H+-Pyr dowex
HO
15
f) CEOP(N(iPr)2)2
CEO—P
N(iPr)2
MMTrN H
2c

Fig. 3

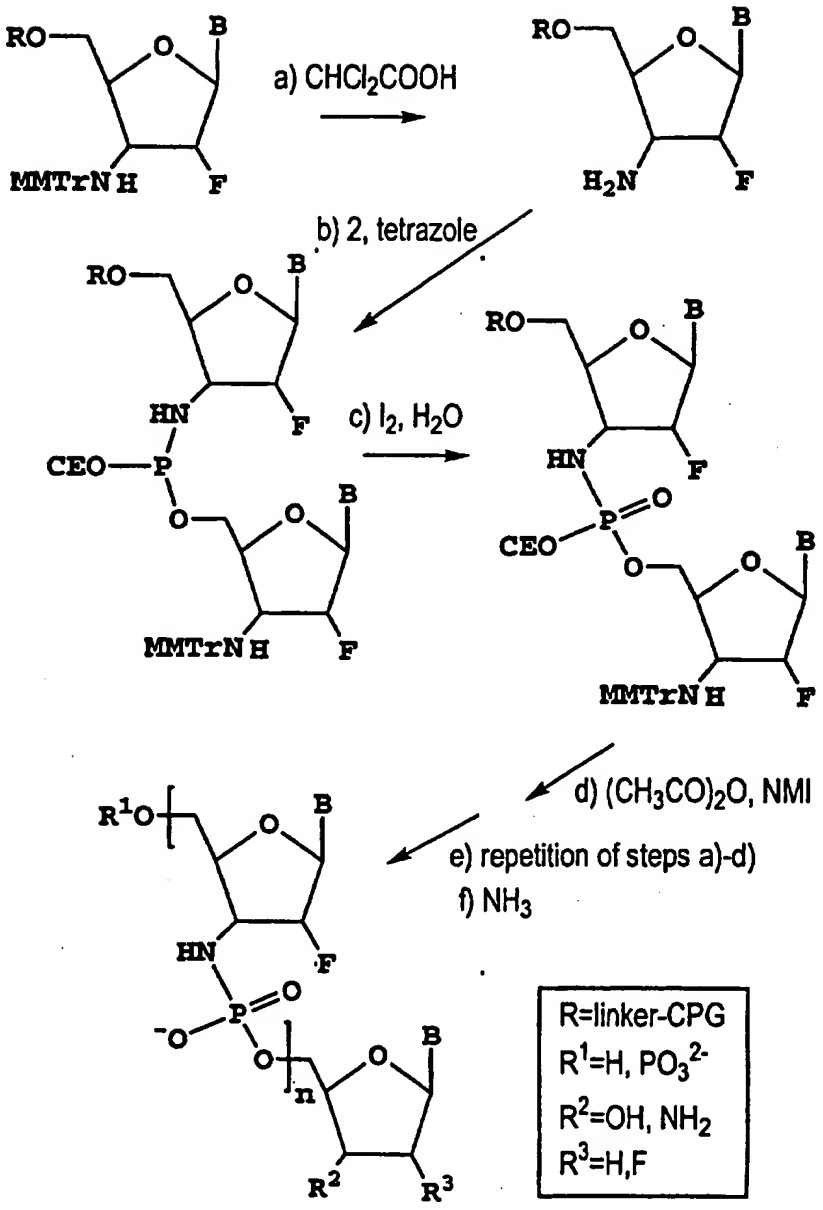

a) $CHCl_2COOH$
b) 2, tetrazole
c) I_2, H_2O
d) $(CH_3CO)_2O$, NMI
e) repetition of steps a)-d)
f) NH_3
R=linker-CPG
R^1=H, PO_3^{2-}
R^2=OH, NH_2
R^3=H,F

Fig. 4

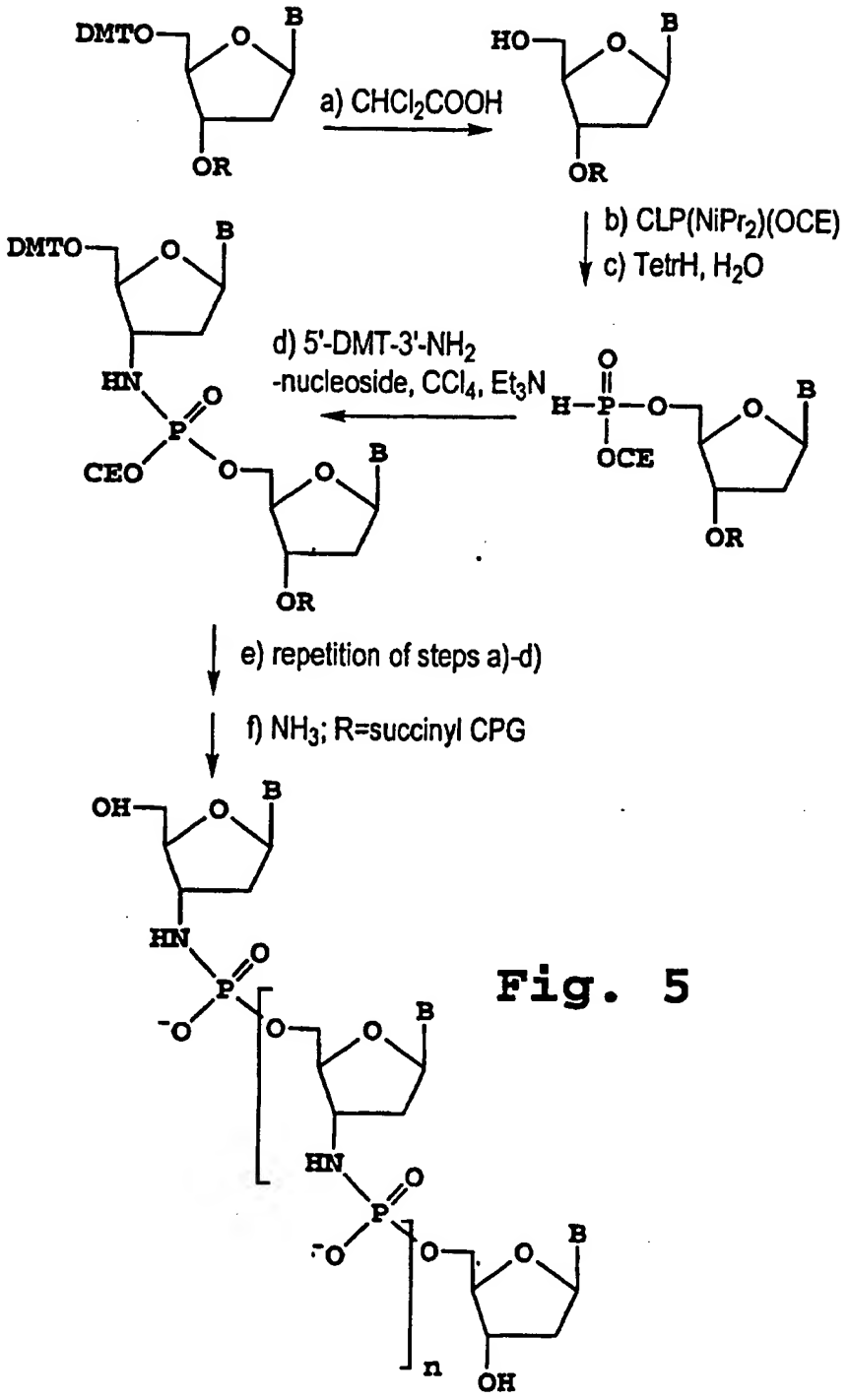

DMTO
B
O
OR
a) $CHCl_2COOH$
HO
b) $CLP(NiPr_2)(OCE)$
c) TetrH, H_2O
d) 5'-DMT-3'-NH_2
-nucleoside, CCl_4, Et_3N
HN
P
CEO
H
OCE
e) repetition of steps a)-d)
f) NH_3; R=succinyl CPG
OH
n

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05773

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00; C07H 21/04
US CL :514/44;536/24.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44;536/24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN, MEDLINE, EMBASE, BIOSIS, WPIDS
search terms: telomerase, inhibit?, oligonucleotide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 5,489,508 A (WEST et al.) 06 February 1996, columns 11-12.	8, 10 ----- 9, 11
Y	GOODCHILD, J. Conjugates of oligonucleotides and modified oligonucleotides: A review of their synthesis and properties. Bioconjugate Chemistry. 1990, Vol. 1, No. 3, pages 165-187, see entire document.	9-11
Y	PIEKEN et al. Kinetic characterization of ribonuclease-resistant 2'-modified hammerhead ribozymes. Science. 19 July 1991, Vol. 253, pages 314-317, see entire document.	9-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 MAY 1997	08 JUL 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	AMY NELSON
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05773

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOSHI et al. Growth inhibitory effects of telomere mimic-oligonucleotides on human leukemia/lymphoma cells in vitro. Proceedings of the American Association for Cancer Research. March 1995, Vol. 36, page 411, No. 2449, see entire abstract.	11